L4 ANSWER 7 OF 16 MEDLINE DUPLICATE 5

AB In the present investigation, effect of rat peripheral polymorphonuclear leukocyte (PMNL) supernatant was investigated on **platelet aggregation**. Rat PMNLs suspended in Hanks balanced salt solution (HBSS, pH 7.4) were incubated at 37 degrees C for different time intervals and cell-free supernatant was obtained by centrifugation. Supernatant was found to inhibit adenosine diphosphate (ADP), arachidonic acid (AA), and calcium ionophore-induced **platelet aggregation**. The inhibitory effect of PMNL supernatant on **platelet aggregation** was not blocked by methylene blue (10 microM) or adenosine deaminase (5 U ml(-1)) pretreatment, suggesting that the inhibitory effect of the supernatant on aggregation was not mediated by nitric oxide (NO) or **ecto-ADPase**. The effect of PMNL supernatant on **platelet aggregation** was abolished by preheating the supernatant at 95 degrees C for 5 minutes. Pretreatment of the supernatant with protease inhibitors abolished the inhibitory effect of supernatant on **platelet aggregation** suggesting that the factor may be a protein or peptide with protease activity. Partial purification of biologically active factor by fine particle liquid chromatography (FPLC) by using Superose 6B column yielded a peak with a molecular weight of approximately 30 kDa having antiaggregatory activity. The results obtained suggest that rat peripheral PMNLs release yet another factor(s) that inhibits **platelet aggregation**. The factor is a heat labile protein with a molecular weight of approximately 30 kDa.

AN 1998387478 MEDLINE

DN 98387478

TI Inhibition of **platelet aggregation** by a protein factor present in rat peripheral polymorphonuclear leukocyte supernatant.

AU Kumari R; Singh M P; Seth P; Dikshit M

CS Division of Pharmacology, Central Drug Research Institute, Lucknow, India.

SO THROMBOSIS RESEARCH, (1998 Jul 15) 91 (2) 75-82.
Journal code: VRN. ISSN: 0049-3848.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199901

File

L4 ANSWER 12 OF 16 MEDLINE DUPLICATE 8
AB Platelet activation by the stable endoperoxide analogue U46619 is mediated
largely by ADP released from platelet-dense granules. Polymorphonuclear leukocytes (PMNs) endowed with **ecto-ADPase** activity may operate as antiaggregatory cells in **platelet aggregation** induced by U46619. Unstimulated PMNs were effective in reducing aggregation when platelets were stimulated by threshold concentrations of U46619, whereas at higher concentrations of the stimulus, PMN activation is required. Evidence that the inhibition was mediated by PMN **ecto-ADPase** activity was obtained by high-performance liquid chromatography analysis, indicating that PMNs were
able to efficiently metabolize platelet-active ADP into AMP. Moreover, PMN-derived supernatants were able to inhibit **platelet aggregation**, suggesting that under this circumstance the inhibition was exerted by an uncharacterized, releasable ADPase activity. This study supports the hypothesis that, besides nitric oxide and hydrogen
peroxide, ADPase activity may represent another PMN-mediated pathway capable of regulating platelet activity in areas of reduced blood flow, such as those found in conditions of myocardial ischemia.

=> d l4 12 bib

L4 ANSWER 12 OF 16 MEDLINE DUPLICATE 8
AN 93250000 MEDLINE
DN 93250000
TI **Platelet aggregation** induced by the endoperoxide analogue U46619 is inhibited by polymorphonuclear leukocyte ADPase activity.
AU Zatta A; Pandolfo L; Caparrotta L; Prosdocimi M; Dejana E; Del Maschio A
CS Fidia Research Laboratories, Abano Terme, Italy..
SO ARTERIOSCLEROSIS AND THROMBOSIS, (1993 May) 13 (5) 696-701.
Journal code: AZ1. ISSN: 1049-8834.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199308

L4 ANSWER 14 OF 16 MEDLINE DUPLICATE 9
AB We previously reported that platelets become unresponsive to agonists when stimulated in combined suspension with aspirin-treated human umbilical vein endothelial cells. Inhibition occurred concomitant with metabolism of platelet-derived endoperoxides to prostacyclin by endothelial cells. We now demonstrate that if aspirin-treated platelets which fully respond to appropriate doses of agonists are exposed to aspirin-treated endothelial cells, they remain unresponsive despite absence of prostacyclin. Platelet inhibition is due in large part to **ecto-ADPase** activity on the endothelial cells. This was established by incubating aspirin-treated endothelial cells with 14C-ADP. Radio-thin layer chromatography and aggregometry demonstrated that 14C-ADP and induction of platelet activation decreased rapidly and concurrently. AMP accumulated transiently, was further metabolized to adenosine, and deaminated to inosine. The apparent Km of the endothelial cell ADPase was 33-42 microM and the Vmax 17-43 nmol/min per 10(6) cells, values in the range of antithrombotic potential. Thus, at least three complementary systems in human endothelial cells control platelet responsiveness: a cell-associated, aspirin-insensitive ADPase which functions in parallel with fluid phase autacoids such as the aspirin-inhibitable eicosanoids, and the aspirin-insensitive endothelium-derived relaxing factor.

=> d l4 14 bib

L4 ANSWER 14 OF 16 MEDLINE DUPLICATE 9
AN 92042759 MEDLINE
DN 92042759
TI Inhibition of platelet function by an aspirin-insensitive endothelial cell ADPase. Thromboregulation by endothelial cells.
AU Marcus A J; Safier L B; Hajjar K A; Ullman H L; Islam N; Broekman M J; Eiroa A M
CS Department of Medicine, Department of Veterans Affairs Medical Center, New York, NY 10010..
NC HL-18828-16 (NHLBI)
HL-47073-01 (NHLBI)
HL-46403 (NHLBI)
+
SO JOURNAL OF CLINICAL INVESTIGATION, (1991 Nov) 88 (5) 1690-6.
Journal code: HS7. ISSN: 0021-9738.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals; Cancer Journals
EM 199202

(360) 546-2497
503 292 7424

AB **CD39**, or vascular adenosine triphosphate diphosphohydrolase, has been considered an important inhibitor of platelet activation. Unexpectedly, **cd39**-deficient mice had prolonged bleeding times with minimally perturbed coagulation parameters. Platelet interactions with injured mesenteric vasculature were considerably reduced in vivo and purified mutant platelets failed to aggregate to standard agonists in vitro. This platelet hypofunction was reversible and associated with purinergic type P2Y1 receptor desensitization. In keeping with deficient vascular protective mechanisms, fibrin deposition was found at multiple organ sites in **cd39**-deficient mice and in transplanted cardiac grafts. Our data indicate a dual role for adenosine triphosphate diphosphohydrolase in modulating hemostasis and thrombotic reactions.

AN 1999401080 MEDLINE
DN 99401080

TI Targeted disruption of **cd39**/ATP diphosphohydrolase results in disordered hemostasis and thromboregulation [see comments].

CM Comment in: Nat Med 1999 Sep;5(9):987-8

AU Enjyoji K; Sevigny J; Lin Y; Frenette P S; Christie P D; Esch J S 2nd; Imai M; Edelberg J M; Rayburn H; Lech M; Beeler D L; Csizmadia E; Wagner D
D; Robson S C; Rosenberg R D
CS Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA.

NC HL57307 (NHLBI)
PO1-41484 (NHLBI)
HL41002
SO NATURE MEDICINE, (1999 Sep) 5 (9) 1010-7.
Journal code: CG5. ISSN: 1078-8956.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199912
EW 19991201

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RB 1/13/00 37

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L4 ANSWER 6 OF 16 MEDLINE DUPLICATE 4
AB Human placental ecto-ATP diphosphohydrolase (ATPDase), an 82 kDa single-chain glycoprotein, was purified to high specific activity using a specific murine monoclonal antibody MK33 (IgG1-kappa). Structurally, protein-based analysis showed this enzyme to be almost identical to that of **CD39** lymphoid cell activation antigen deduced by cDNA sequencing (Maliszewski CR, et al, J Immunol 1994; 153:3574); but differing in the NH2-terminal amino acid sequence, suggesting that placental ecto-ATPDase is most likely an isoform of **CD39** generated by alternative splicing of the pre-mRNA. Functionally, placental ecto-ATPDase totally inhibits the secondary **platelet aggregation** induced by agonists at a final concentration (f.c.) of 1 microgram/ml. The purified enzyme (1 microgram/ml, final), pre-incubated with washed platelets prior to alpha-thrombin stimulation, completely inhibits the activation of platelet glycoprotein (GP) IIb/IIIa, thereby blocking the binding of fibrinogen or von Willebrand factor to platelets. Further, under different shear stresses, the enzyme modulates **platelet aggregation** differently. Low shear stress-induced **platelet aggregation** is blocked by this enzyme in a dose-dependent manner and is totally blocked at f.c. 0.5 microgram/ml. Under high shear stress, however, this protein at a f.c. of 0.5 microgram/ml mediates almost complete disaggregation of platelets without affecting the initial aggregation. Using immunohistochemical analysis, this enzyme was observed to be localized at the syncytiotrophoblasts of placental microvilli and the endothelial cells (ECs) of the umbilical vein obtained at full-term normal delivery, but scarcely at the ECs of the umbilical artery.

=> d 14 6 bib

L4 ANSWER 6 OF 16 MEDLINE DUPLICATE 4
AN 1999062444 MEDLINE
DN 99062444
TI Placental ecto-ATP diphosphohydrolase: its structural feature distinct from **CD39**, localization and inhibition on shear-induced **platelet aggregation**.
AU Makita K; Shimoyama T; Sakurai Y; Yagi H; Matsumoto M; Narita N; Sakamoto Y; Saito S; Ikeda Y; Suzuki M; Titani K; Fujimura Y
CS Department of the 2nd Internal Medicine of Nara Medical University, Japan.
SO INTERNATIONAL JOURNAL OF HEMATOLOGY, (1998 Oct) 68 (3) 297-310. Journal code: A7F. ISSN: 0925-5710.
CY Ireland
DT Journal; Article; (JOURNAL ARTICLE)
LA English
EM 199903
EW 19990301

in mouse model ?.

order

L4 ANSWER 2 OF 16 MEDLINE DUPLICATE 1

AB The human ecto-apyrase gene family consists of five reported members (**CD39**, **CD39**-L1, **CD39**-L2, **CD39**-L3, and **CD39**-L4). The family can be subdivided into two groups by conservation of proposed structural domains. The **CD39**, **CD39**-L1, and **CD39**-L3 genes all encode hydrophobic portions in their carboxy and amino termini, serving as transmembrane domains for **CD39** and potentially for the other two members. **CD39**-L2 and **CD39**-L4 genes encode hydrophobic portions in their amino termini, suggesting that they might encode secreted apyrases. We demonstrate that the **CD39**-L4 gene encodes the first reported human secreted ecto-apyrase. COS-7 cells transfected with a **CD39** -L4 expression construct utilizing the naturally occurring leader peptide express recombinant protein outside of the cells. This expression can be blocked by brefeldin A, a chemical that inhibits a step in mammalian secretory pathways. We also demonstrate expression of **CD39**-L4 message in macrophages, suggesting that the protein is present in the circulation. Furthermore, we show that **CD39**-L4 is an E-type apyrase, is dependent on calcium and magnesium cations, and has high degree of specificity for NDPs over NTPs as enzymatic substrates. A potential physiological role in hemostasis and **platelet aggregation** is presented.

=> d l4 2 bib

L4 ANSWER 2 OF 16 MEDLINE DUPLICATE 1

AN 1999329001 MEDLINE

DN 99329001

TI **CD39**-L4 is a secreted human apyrase, specific for the hydrolysis of nucleoside diphosphates.

AU Mulero J J; Yeung G; Nelken S T; Ford J E

CS Functional Genomics Department, Hyseq Inc., Sunnyvale, California 94086, USA.

SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Jul 16) 274 (29) 20064-7. Journal code: HIV. ISSN: 0021-9258.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals; Cancer Journals

EM 199910

EW 19991003

fragment
102a

L4 ANSWER 11 OF 16 MEDLINE DUPLICATE 7
AN 97115858 MEDLINE
DN 97115858
TI Identification and characterization of **CD39**/vascular ATP diphosphohydrolase.
AU Kaczmarek E; Koziak K; Sevigny J; Siegel J B; Anrather J; Beaudoin A R; Bach F H; Robson S C
CS Sandoz Center for Immunobiology, New England Deaconess Hospital, Harvard Medical School, Boston, Massachusetts 02215, USA.. srobson@nedhmail.nedh.harvard.edu
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Dec 20) 271 (51) 33116-22. Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
OS GENBANK-U87967
EM 199703

=> d l4 11 ab

L4 ANSWER 11 OF 16 MEDLINE DUPLICATE 7
AB Vascular ATP diphosphohydrolase (ATPDase) is a plasma membrane-bound enzyme that hydrolyses extracellular ATP and ADP to AMP. Analysis of amino acid sequences available from various mammalian and avian ATPDases revealed their close homology with **CD39**, a putative B-cell activation marker. We, therefore, isolated **CD39** cDNA from human endothelial cells and expressed this in COS-7 cells. **CD39** was found to have both immunological identity to, and functional characteristics of, the vascular ATPDase. We also demonstrated that ATPDase could inhibit **platelet aggregation** in response to ADP, collagen, and thrombin, and that this activity in transfected COS-7 cells was lost following exposure to oxidative stress. ATPDase mRNA was present in human placenta, lung, skeletal muscle, kidney, and heart and was not detected in brain. Multiple RNA bands were detected with the **CD39** cDNA probe that most probably represent different splicing products. Finally, we identified an unique conserved motif, DLGGASTQ, that could be crucial for nucleotide binding, activity, and/or structure of ATPDase. Because ATPDase activity is lost with endothelial cell activation, overexpression of the functional enzyme, or a truncated mutant thereof, may prevent platelet activation associated with vascular inflammation.

QP501.J7

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L7 ANSWER 9 OF 32 MEDLINE DUPLICATE 3

AB The glycoprotein (GP) IIb/IIIa (the alphallb beta3 integrin) found on platelets binds fibrinogen or von Willebrand factor when the platelet is activated, thereby mediating the aggregation of platelets. Blockade of the GPIIb/IIIa should prevent **platelet aggregation** independent of the substance or substances responsible for activating the platelets. This comprehensive inhibition of **platelet aggregation** is thought to be an effective therapeutic approach to various clinical thromboembolic syndromes. This study investigated the platelet inhibition provided by blocking GPIIb/IIIa by using a new nonpeptidic molecule, BIBU52, in both in vitro and in vivo models. BIBU52 competes with [125I]fibrinogen for binding sites on human platelets in a Ca2+ and pH-dependent manner with a 50% inhibitory concentration (IC50) of 35 +/- 12 nM. BIBU52 inhibited the aggregation of human platelets in platelet-rich plasma induced by collagen (1-2 microg/ml), adenosine diphosphate (**ADP**; 2.5 microM), and a thrombin receptor-activating peptide (TRAP; SFLLRNPNDKYEPF-NH2; 25 microM) with IC50 values of 82, 83, and 200 nM, respectively. The inhibition of **platelet aggregation** by BIBU52 was found to be highly species dependent. BIBU52 inhibited aggregation in plasma from rhesus and marmoset monkeys with an IC50 of 150 nM but was totally ineffective in rat plasma. The selectivity of BIBU52 for inhibiting GPIIb/IIIa in comparison with other adhesion molecules was investigated in a human endothelial cell adhesion assay. The adhesion of human endothelial cells to matrices of vitronectin, fibronectin, collagen I, or laminin was not affected by concentrations as high as 100 microM BIBU52; thus BIBU52 demonstrates a high selectivity for the human GPIIb/IIIa.The antithrombotic effect of BIBU52 in vivo was investigated in three **animal models** of recurrent arterial thrombus formation. In the guinea pig aorta, BIBU52 inhibited thrombus formation dose dependently, with lack of thrombus formation for 1 h after a bolus dose of 1.0 mg/kg i.v.. Both acetylsalicylic acid and dazoxiben were less effective in this model. In pigs with recurrent thrombus formation in the carotid artery, 1.0 mg/kg i.v. also inhibited thrombus formation. Heparin was not effective in the pig, and acetylsalicylic acid was only partially effective. In the pig, the dose of 1.0 mg/kg i.v. BIBU52 also was associated with a 70% inhibition of collagen-induced **platelet aggregation** ex vivo but with only a transient prolongation of sublingual bleeding time to a maximum of 2.5-fold and without other hemodynamic effects. In the marmoset monkey, a dose of 10 microg/kg i.v. could abolish recurrent arterial **thrombosis**. Hemodynamic effects of BIBU52 in anesthetized pigs were not detected in doses < or = 10 mg/kg. These data demonstrate that BIBU52 is a potent and selective antagonist of the human GPIIb/IIIa receptor and capable of substantial inhibition of **platelet aggregation** in vitro and ex vivo as well as inhibition of arterial thrombus formation in vivo in **animal models** of **thrombosis**.

=> d l7 9 bib

L7 ANSWER 9 OF 32 MEDLINE DUPLICATE 3

AN 97413541 MEDLINE
DN 97413541
TI Antagonism of the GPIIb/IIIa receptor with the nonpeptidic molecule BIBU52: inhibition of **platelet aggregation** in vitro and antithrombotic efficacy in vivo.
AU Guth B D; Seewaldt-Becker E; Himmelsbach F; Weisenberger H; Muller T H
CS Department of Biological Research, Dr. Karl Thomae GmbH, Biberach an der Riss, Germany.
SO JOURNAL OF CARDIOVASCULAR PHARMACOLOGY, (1997 Aug) 30 (2) 261-72. Journal code: K78. ISSN: 0160-2446.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199712
EW 19971201